PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference FPAA/288 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IN 03/00204	International filing date *(day/month/year)* 30.05.2003	Priority date *(day/month/year)* 31.05.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant SECRETARY, DEPARTMENT OF ATOMIC ENERGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 20 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.12.2003	28.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Leber, T Telephone No. +49 89 2399-7195

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IN 03/00204

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-66 as originally filed

Sequence listings part of the description, Pages

67-81 as originally filed

Claims, Numbers

1-56 received on 11.08.2004 with letter of 08.08.2004

Drawings, Sheets

1/38-38/38 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 56

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 56 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-37,40-43,45-47,49-55
	No:	Claims	44,48
Inventive step (IS)	Yes:	Claims	1-36
	No:	Claims	37,40-55
Industrial applicability (IA)	Yes:	Claims	1-55
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the opinion

1. The sequence listing pages 1-9 filed with the letter of 05.02.2004 does not form part of the application (Rule 13ter.1(f) PCT).

2. With letter dated 09.08.2004, the Applicant filed amended claims 1-56 to replace the previous set of claims on file. Claims 37-39 appear to go beyond the application as originally filed (see below) and the amendments are consequently not considered to have been made (Art 70.2(c) PCT).

   Amended claim 37 refers to a kit encompassing at least two oligonucleotides as a pair of primers for amplification of a target sequence such that after amplification the 3' ends of the said pair of primers are on two opposite strands and separated from one another by 0-25 nucleotide pairs in the final amplification product. - The application as originally filed appears not to provide a basis for the feature "at least two oligonucleotides" or for the feature of the distance of "0-25 nucleotide pairs" in the context of a kit. Claim 37 therefore fails to comply with Art 34(2)(b) PCT. The same objections apply to dependent claim 38 (Art 34(2)(b) PCT).

   Claim 39 refers to a kit whereby the donor and/or acceptor MET entity on the oligonucleotide primer is provided quenched. The application as originally filed appears not to provide a basis for this general feature in the context of a kit (Art 34(2)(b) PCT) but only for particular ways of quenching (see, for example, claim 43 as originally filed).

   In view of the objections raised to claims 37-39 in item 2. above, claim 37 is examined not considering the amendments introduced and thus in the wording of claim 42 as originally filed from which claim 37 on file was derived. With regard to claims 38 and 39, no examination was carried out with regard to novelty, inventive step and industrial applicability as the said claims could not be related to unamended claims lacking the features objected to above.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability.

1. Claim 56 refers to a "method for the detection of a target nucleic acid sequence, a kit used for the same and its process of manufacture substantially as herein described and illustrated with reference to the examples and figures and many modifications thereof".
The said definition is so unclear (Art 6 PCT), that no meaningful examination can be carried out (Art 34(4)(a)(ii) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Basis for the assessment of novelty, inventive step and industrial applicability

1.1 Reference is made to the following document/s/:

D1: US-A-5 866 336 (NAZARENKO IRINA A ET AL) 2 February 1999 (1999-02-02)
D2: US-B-6 287 7811 (LEE MARTIN ALAN ET AL) 11 September 2001 (2001-09-11)

1.2 The amendments filed with the letter of 09.08.2004 do not fulfill the requirements of Art 34(2)(b) PCT (see Item I 2., above).

1.3 Products must be defined by technical features (Rule 6.3 PCT). The oligonucleotides referred to in claims 37, 44 and 45 are defined by the result to be achieved only, namely by binding at a particular position with regard to the target nucleotide. The target nucleic acid, however, is not part of the kit. The definition of the said claims is therefore unclear as the skilled person cannot distinguish whether or not particular oligonucleotides fall under the scope of the said claims (Art 6 PCT; Rule 6.3 PCT; Guidelines, Section IV, III-4.7).
The said definitions are therefore not regarded as limiting features of the said claims for the examination presented below.

1.4 The reference in a kit-claim to a method is understood as an indication that the kit is merely **suitable** to carry out the said method. The kit may, however, also be used for other methods. Consequently, a kit cannot be regarded as being novel and inventive for the sole reason that the method to which it refers is novel and inventive (PCT Guidelines, Section IV, III-4.8).

2. Novelty and inventive step

2.1 Claim 1 appears to be novel over D1 (Art 33(2) PCT). Dependent claims 2-36 are thus also novel (Art 33(2) PCT).

2.2 Document D1 discloses an amplification method whereby two oligonucleotides are involved which are labelled with a donor and acceptor fluorescent label, respectively, and which are homologous to complementary strands. Incorporation of the labelled oligonucleotides in an amplification product results in FRET between the said fluorescent labels (D1, col. 9, lines 53-60; Fig. 7; col. 25, line - col. 26, line 12). The method is suitable for direct monitoring of the amplification reaction (D1, col. 4, lines 35-42; col. 8, lines 26-38) and one of the oligonucleotides is a primer whereas the other is a probe (D1, Fig. 7). The target nucleic acid may be RNA (D1, col. 19, line 22). Document D1 moreover discloses a kit for tri-amplification encompassing two oligonucleotides labelled with a donor/acceptor moiety, respectively (D1, col. 33, lines 12-31). Thus, claims 44 and 48 lacks novelty over D1 (Art 33(2) PCT).

2.3 Claim 1 differs from closest prior art document D1 in that (I) the two oligonucleotide primers are labelled with donor and acceptor moiety, respectively, and (ii) in that the said primers are designed in such a way that when incorporated into the amplification product, their 3' ends are 0-25 bp apart.
The technical effect resulting from this difference appears to be that the method is simpler and cheaper as there is no need for a third oligonucleotide as in D1 (see D1, "tri-amplification"; Fig. 7) and as the method requires only a polymerase whereas the tri-amplification referred to in D1, requires both a ligase and a polymerase.

The technical problem may thus be formulated as the provision of an simplified nucleic acid detection method avoiding the need for a third oligonucleotide and a ligase as required for tri-amplification.

The solution provided by claim 6 resides in the design of the donor/acceptor labelled primers to take a position in the amplification product such that their 3' ends are 0-25 bp apart allowing FRET/MET to occur.

It appears that an inventive step can be acknowledged for this solution as none of the available documents provides an indication for the skilled person to solve the above defined technical problem by the said solution (Art 33(3) PCT). Dependent claims 2-36 are thus also inventive (Art 33(3) PCT).

2.4 Claim 37 (worded as 42 as originally filed; see item I 2. above) appears to be novel over the available prior art (Art 33(2) PCT). Claim 37 differs from closest prior art document D1 in the absence of (I) a reaction buffer, (ii) deoxy nucleotides, (iii) a polymerase and (iv) in the definition that the fluorescent label is at or near the 3' end.
It appears that the differences (I)-(iii) per se cannot form a basis for an inventive step (Art 33(3) PCT) as it belongs to the routine of the skilled person to determine which of the reagents needed are incorporated into the kit and which the user has to provide. A commercially available PCR-kit, for example, will contain the products (I)-(iii) but not the thermal cycler or other standard means required to carry out the PCR method (e.g. tips, water etc.). The fourth difference appears not to represent a solution to a technical problem but a random selection of a particular section of the oligonucleotide to be labelled (Art 33(3) PCT). Claim 47 lacks an inventive step for the same reasons (Art 33(3) PCT).

2.5 Claim 45 appears to be novel over the available prior art (Art 33(2) PCT). The said claim 45 differs from closest prior art document D2 in two fluorescently labelled oligonucleotides are present (see D2, Fig. 1; claim 21). The technical effect resulting from this difference appears to be that two target molecules can be detected.
The technical problem may therefore be formulated as the provision of a kit for the detection of more target molecules.
It appears that the solution provided in claim 45, namely to add a further labelled oligonucleotide to the kit, is trivial as the skilled person knows that with each oligonucleotide probe, a different target molecule can be detected. Thus, no inventive step can be acknowledged (Art 33(3) PCT).

2.6 Dependent claims 40-43, 46, 49-55 do not contain any features which, in combination with the features of any claim to which they refer, meet the require-

ments of the PCT in respect of inventive step as they refer to subject-matter that is routinely applied by the skilled person in molecular biology related to amplification reactions taking advantage of molecular or fluorescence energy transfer effects.

4. Industrial applicability

4.1 The subject-matter disclosed in the claims 1-55 of the present application appears to be industrially applicable (Art 33(4) PCT).